Notes on the Natural History and Reproductive Biology of *Isoëtes malinverniana*.—*Isoëtes malinverniana* Cesati *et* De Notaris is a narrowly endemic species from the administrative districts of Lombardy and Piedmont in north-west Italy. To date only twelve populations of this plant grow in a heavily disturbed habitat in the plain of the river Po. Water channels where this plant lives are under the constant threat of nutrient enrichment and pesticide contamination deriving from their use as water supply for rice crops. This is one of the main reasons for the species was listed as Critically Endangered in the Italian IUCN Red-List (Conti *et al.*, Università di Camerino. 1997) and is presently under study for the aims of conservation.

Isoëtes malinverniana is a tetraploid species ($2n = 44$; Löve *et al.*, Cytotaxonomical atlas of the Pteridophyta. J. Cramer, Vaduz. 1977) of uncertain origin that is genetically distant from all the other species in the genus. The closest relatives are some species (i.e., *I. coromandelina* L.f.) from the south-east Asia (Hoot *et al.*, Syst. Bot. 31:449–460. 2006). Here we give some insights on the reproductive biology and phenology of *I. malinverniana* as a result of field and laboratory observations for the sake of addressing future conservation efforts.

In 2010, germination tests were run with *I. malinverniana* spores at the Lombardy Seed Bank of the University of Pavia (LSB), with the aim to test for the self-compatibility of the species. Since *I. malinverniana* is very rare, the experiment was limited to two plants, to avoid affecting the survival of the source population which is located in a branch of the Cavo Bogino Channel, a few hundred meters north of the town of Vigevano, in the Lombardy district. Specimens used as a source of spores in this study were cultivated at the Botanical Garden of the University of Pavia.

Megaspores from the two plants were sown on 1% agar after wetting the surface of the substrate with sterile water. A total of 134 megaspores (64 and 70 from plant A and B, respectively) were plated into two different Petri dishes. Megaspores from plant A were fertilized adding microspores from plant A and the same procedure was followed for plant B using its own microspores. Spores were incubated at 20°C in light (12/12h day/night) and checked weekly for the emergence of sporelings. The experiment ended after four weeks when all the megaspores had germinated.

Sporophyte production was rapid, resulting in 100% and 99% emergence from megaspores from plants A and B, respectively, within the fourth week. These reproductive rates are very high when compared with other *Isoëtes* species in culture. Depending on the treatment, Kott and Britton (Can. J. Bot. 60:679–1687. 1982) obtained on average 87–89% megaspores emitting sporophytes for *I. macrospora* Durieu, 58–63% for *I. tuckermanii* A.Braun, 2–39% for *I. riparia* Engelm., 0–17% for *I. acadiensis* Kott and 0–15% for *I. echinospora* Durieu.

Our results seem to rule out the self-incompatibility of this species reported by Schneller (Webbia 35: 307–309. 1982), who noted that in nature megasporangia of *I. malinverniana* mature well before microsporangia and that this lack of synchrony is expected to increase the chance of cross

fertilization. Our observations, gathered in the field during the whole year between 2008 and 2009 on wild populations and cultivated plants revealed that in *I. malinverniana* development of microsporangia precedes that of megasporangia. The latter were present from January to June and localized in the innermost leaves. Some mature megaspores were already visible at the end of January. Microsporangia were localized in the outermost leaves and matured earlier in both wild and cultivated plants starting from the end of December onward. Our observations are in contrast with other authors who reported that spore dispersal for *I. malinverniana* begins between summer and autumn (Pignatti, Edagricole Bologna. 1982; Marchetti, Ann. Mus. Civ. Rovereto 19:71–231. 2003). A possible explanation for this discrepancy may be found in the possibility to find some mature megaspores until October. Despite the high viability of the spores, sporelings of *I. malinverniana* are short-lived in laboratory and they often die in the first three months. Even in the field, the observation of young plants is very rare. The causes are still unknown, but probably involve both genetic and ecological factors. In some sites the water flow was very fast and can be a cause of up-rooting of the sporelings.

Eighteen sporelings grown in an aquarium very close to the mother plant were dug immediately after their emergence to determine if they were the product of sexual or vegetative reproduction. The presence of a megaspore at their base excluded a vegetative origin from the mother plant, but we cannot exclude apogamy. Vegetative reproduction is known in *I. tegetiformans* Rury (Rury, Amer. Fern J. 68:99–108. 1978), *I. storkii* T.C.Palmer (Gomez, Brenesia 18:1–14. 1980), *I. lechleri* Mett. (Hickey, Fieldiana (Bot) NS 34:88–97. 1994), *I. andicola* (Amstutz) L.D.Gómez (Karrfalt, Amer. Fern J. 89:198–203. 1999) and *I. sinensis* Palmer (Chen *et al.*, Biodiversity Science 12:564–571. 2004), but to date, no evidence of this ability has been observed in *I. malinverniana*.

Our results provide new and important insights in *I. malinverniana* ecology. Firstly, we have more clearly elucidated the reproductive phenology of this species showing that megaspores and miscrospores develop during the winter, through the spring and until the early summer. Through culture experiments, we have also found that megaspores germinate promptly and rapidly produce sporophytes, and are highly self compatible, a likely event given the contemporary presence of both kinds of spores.

Recent AFLP and ISSR analysis (Gentili *et al.*, Aquat. Bot. 93:147–152. 2010) revealed high genetic variability within *I. malinverniana* populations in comparison to other species in the genus. According to the authors this could be a consequence of the out-breeding behavior of the species and of its high level of ploidy. In light of our results, the latter explanation seems more important for the maintenance of genetic variability and it could also play a role in enforcing the viability and germination ability of the spores (Kott and Britton, 1982).

The rapid emergence of the sporelings we obtained at 20°C, suggest that *I. malinverniana* megaspores do not require a cold stratification to germinate. Incubation at low temperatures has been reported to increase germination in

other species such as *I. echinospora*, *I. riparia* and *I. acadiensis* (Kott and Britton, 1982). In *I. malinverniana,* however, recruitment of megaspores through winter stratification deserves confirmation and new experiments aimed to test cold stratification in cultured spores are ongoing.—THOMAS ABELI, DISTA, Dipartimento di Scienze della Terra e dell'Ambiente, University of Pavia, via S. Epifanio 14, 27100, Pavia, Italy, e-mail: thomas.abeli@unipv.it, and MARCO MUCCIARELLI, Dept. of Veterinary Morphophysiology, University of Torino, via Leonardo da Vinci 44, 10095, Grugliasco (TO), Italy, e-mail: marco.mucciarelli@unito.it.